METHODS FOR TREATING SKIN CONDITIONS

This is a continuation-in-part application of U.S. Patent Application Serial No. 09/110,409 (Attorney Docket No. JBP 430) filed July 6, 1998, and U.S. Patent Application Serial No. 09/698,454 (Attorney Docket No. JBP 518) filed October 27, 2000, which are hereby incorporated herein by reference.

1. Field of the Invention

This invention is related to methods and compositions for treating and ameliorating skin conditions including acne, rosacea and wrinkling caused by photodamage or intrinsic aging. More particularly, this invention relates to compositions containing certain natural extracts and natural or synthetic retinoids.

2. Background of the Invention

Acne is an inflammatory dermatological disorder, which occurs frequently in adolescence and with some regularity in older adults of the human species. The condition of acne can include skin lesions ranging from the comedo in a pilosebaceous follicle, to more severe como-inflammatory symptoms such as pustules, papules, cysts and nodules. The condition is not only uncomfortable for the victim, but also embarrassing, and can result in disfigurement and scarring.

The pathology of acne vulgaris is believed to involve a number of actors: the first of which is the formation of comedones more commonly referred to as whiteheads (closed comedones) and blackheads (open comedones). These are solid horny masses that plug follicles and are associated with increased production of sebum. They are made up of tightly packed keratinized cells and sebum. As the comedo enlarges through continued accumulation of keratinized cells, pressure builds up within the follicles which eventually rupture, dumping the contents consisting of horny material, sebum and bacteria

into the skin. This provokes inflammatory responses which take the form of pustules (pimples) when the rupture is small and cystic-nodules with complete rupture.

Many different approaches to ameliorating this disorder have been attempted in the past, with some treatments more effective than others. Attacks ranging from simple washing and cleansing to pharmaceuticals have been employed. One group of agents used in acne treatment include the retinoids and retinols. While these agents can significantly improve acne, their undesirable side effects range from mild to severe irritation, redness, peeling, and itching and burning sensation. Thus, it is desired to have a single topical treatment that could prevent or reverse acne with minimal or no undesired side effects.

Aging of the skin is a complex phenomenon resulting from the interaction of several intrinsic and extrinsic factors. Skin changes associated with aging often manifest as cosmetic disabilities. Due to its psychological impact, aging of the skin has become an issue of great social significance and concern. With baby boomers aging, the era of cosmetic care, cosmetic maintenance and rejuvenation gains increased awareness. Methods for preventing and treating skin aging are highly desired. Intrinsic aging is an inevitable, genetically programmed process. Among extrinsic influences (wind, heat, cigarette smoke, chemicals, etc.), ultraviolet radiation appears to be the single most important factor associated with aging of the skin. Photoaging is induced by cumulative exposure to ultraviolet radiation (UVR). Increased recreational sun exposure, including excessive sunbathing, the depletion of stratospheric ozone, and the use of UVR in the treatment of various skin diseases, have led to increased prevalence of photoaging during the last decades. Photodamage can be prevented by sun avoidance and proper sun protection, and could be reversed by the use of topical retinoids, which could be irritating and expensive. Overexposure to ultraviolet and visible radiation also causes sunburn. The use of aspirin and other nonsteroidal anti-

inflammatory drugs, cool baths and topical steroids offer only mild relief.

Various approaches to treating acne, photodamage and other skin conditions have been attempted in the past, including treatment with Vitamin A acid (also known as "tretinoin") and natural retinoids or retinoid precursors such as Vitamin A alcohol (also known as "retinol"). (See U.S. Patent No. 4,877,805 and U.S. Patent No. 4,355,028, for example). However, topical treatment with retinoids can be very irritating to the skin and uncomfortable for the patient. It can cause redness, which may be embarrassing to the patient, particularly those suffering from acne in their teenage years. Oral treatment with retinoids has been found to have teratogenic effects.

Thus, it would be desirable to find a topical treatment for acne, rosacea, photodamage and other skin conditions that does not cause redness to the skin.

Summary of the Invention

In accordance with this invention, we have found compositions and methods for treating and ameliorating acne, rosacea, wrinkles and photodamage containing nondenatured plant extracts including legume and vegetable extracts having trypsin inhibitory activity and a natural or synthetic retinoid or retinol compounds.

Detailed Description of the Preferred Embodiments

Preferably, the compositions of this invention contain nondenatured legume or vegetable extracts containing compounds that inhibit trypsin, such as serine protease inhibitors. In particular, nondenatured legume extracts will also be useful in methods of this invention. More preferably, nondenatured soybean, limabean and blackbean extracts, and other natural products made from these beans, such as, but not limited to, bean milk, bean paste, and the like, also serve to reduce pigmentation by this mechanism. Serine protease inhibitors isolated from vegetables or legumes are also useful in

this invention, such as, but not limited to, the soybean-derived proteins soybean trypsin inhibitor, "STI" and Bowman-Birk Inhibitor, "BBI".

The novel compositions of this invention preferably contain legume products, and more preferably, soy products, that may be in the form of a fluid (e.g., soymilk) or a solid (e.g., a soybean powder or soymilk powder). What is meant by "soy product" is a substance derived from the soybean, containing the ingredients naturally found in soybeans, at the relative concentrations as found in the beans. What is meant by a "Soy Product" is a substance derived from the soybean. The soy product may contain only a portion of the soybean (e.g., an extract of the soybean such as a lipid reduced soybean powder or filtered soymilk) or may contain the entire soybean (e.g., a ground powder of the legume). The soy product may be in the form of a fluid (e.g., soymilk) or a solid (e.g., a soybean powder or soymilk powder). When in the form of a fluid, the term "soy product" refers to the solid constituents of the fluid that are derived from the soybean.

The soy product may be soybean powder. Soybean powder may be made by grinding dry soybeans. The soybean powder may be lyophilized. Soymilk and soymilk powder are also useful soy products. Soymilk is a combination of solids derived from soybeans and water, the mixture of which has some or all of the insoluble constituents filtered off. Soymilk powder is evaporated soymilk, which in one embodiment, is in a lyophilized or spray-dried form. Procedures for manufacturing soymilk include, but are not limited to, the following three procedures. First, soymilk may be made by placing soybeans into water to allow them to absorb the water. The swelled beans are then ground and additional water is then added. The mixture may then be filtered to remove any insoluble residue. Second, soymilk may also be prepared from soybean powder. Soybean powder is thoroughly mixed with water (e.g., for at least one hour), which may then be followed by a filtration process to remove insoluble residues. Third, soymilk can also be reconstituted from

soymilk powder by adding water. The soymilk may comprise from between about 1% to about 50%, by weight (e.g., from about 5% to about 20%, by weight) of solids from the soybean.

The surface of legume fruits often contain high levels of microorganisms. Thus, prior to use by humans, the legume product needs to be treated to reduce or eliminate such microorganisms.

The legume products utilized in the present invention may have a total microbial content of less than about 10,000 colony-forming units ("cfu") per gram. Preferably, the soy products utilized in the present invention have a microbial content of less than about 1,000 cfu per gram (such as less than about 100 cfu per gram) of the legume product.

The legume products utilized in the present invention may have a total objectionable microbial content of less than 300 cfu per gram such as less than 150 cfu per gram. Preferably, the legume products utilized in the present invention have an undetectable amount of any objectionable microbials for at least one gram (e.g., at least ten grams) of legume product.

The legume product may be exposed to gamma irradiation. The legume product may be exposed to between about 2 to about 30 kGy of gamma irradiation, such as between about 5 and about 10 kGy of gamma irradiation. Such treatment reduces the microbial content of the legume product, while maintaining its biological activity (e.g., serine protease inhibitory activity). The treatment of legume products with gamma irradiation maintains the cosmetic elegance of the legume product, such as maintained natural colors and does not induce significant malodors.

Other anti-microbial processes that also maintain the protease inhibitory activity of the legume product that can be practiced alone or in combination with gamma irradiation, include, but are not limited to, exposure to x-rays, high energy electron or proton beams, ultraviolet radiation, hydrostatic pressure, and addition of chemical agents possessing antimicrobial activity, and combinations thereof.

In one embodiment, the soy product is a non-denatured soy

product. "Denaturation" is defined in the Bantam Medical Dictionary (1990 edition) as "the change in the physical and the physiological properties of a protein, that are brought about by heat, X-rays or chemicals. These changes include loss of activity (in the case of enzymes) and loss (or alteration) of antigenicity (in the case of antigens)". What is meant by "non-denatured plant extract" is a product extracted or derived from a plant in which the processing for the derivation of such plant extract (e.g., the temperature, extraction media) did not eliminate its protease inhibitory activity. One such protease is trypsin. In one embodiment, the non-denatured state of the soy product of this invention is measured by the presence of an intact soybean trypsin inhibitor (STI) protein, or by its trypsin inhibitory activity.

Additional sources of serine protease inhibitors may be extracted from the species belonging to the following plant families: Solanaceae (e.g., potato, tomato, tomatilla, and the like); Gramineae (e.g., rice, buckwheat, sorghum, wheat, barley, oats and the like); Cucurbitaceae (e.g., cucumbers, squash, gourd, luffa and the like); and, preferably, Leguminosae (e.g., beans, peas, lentils, peanuts, and the like). Ingredients in soy, such as isoflavones, or soy trypsin inhibitor, or non-denatured soy have not previously been known or utilized for reducing retinoid-induced irritation or redness. Surprisingly, we have found that compositions containing such elements are capable of reducing retinoid-induced irritation or redness without affecting retinoid activity.

The compounds which are active in the compositions and methods of this invention may be delivered topically by any means known to those of skill in the art. If the delivery parameters of the topically active pharmaceutical or cosmetic agent so require, the topically active composition of this invention may preferably be further composed of a pharmaceutically or cosmetically acceptable vehicle capable of functioning as a delivery system to enable the penetration of the topically active agent into the skin.

One acceptable vehicle for topical delivery of some of the compositions of this invention, particularly proteins such as trypsin and STI, may contain liposomes. The liposomes are more preferably non-ionic and contain a) glycerol dilaurate (preferably in an amount of between about 5% and about 70% by weight); b) compounds having the steroid backbone found in cholesterol (preferably in an amount of between about 5% and about 45% by weight); and c) one or more fatty acid ethers having from about 12 to about 18 carbon atoms (preferably in an amount of between about 5% and about 70% by weight collectively), wherein the constituent compounds of the liposomes are preferably in a ratio of about 37.5:12.5:33.3:16.7. Liposomes comprised of glycerol dilaurate / cholesterol/ polyoxyethylene -10-stearyl ether/polyoxyethylene-9-lauryl ether (GDL liposomes) are most preferred. Preferably the liposomes are present in an amount, based upon the total volume of the composition, of from about 10 mg/mL to about 100 mg/mL, and more preferably from about 20 mg/mL to about 50 mg/mL. A ratio of about 37.5:12.5:33.3:16.7 is most preferred. Suitable liposomes may preferably be prepared in accordance with the protocol set forth in Example 1 of parent application U.S. Serial No. 09/110,409, though other methods commonly used in the art are also acceptable. The above described composition may be prepared by combining the desired components in a suitable container and mixing them under ambient conditions in any conventional high shear mixing means well known in the art for non-ionic liposomes preparations, such as those disclosed in Niemiec *et al.*, "Influence of Nonionic Liposomal Composition On Topical Delivery of Peptide Drugs Into Pilosebacious Units: An In Vivo Study Using the Hamster Ear Model," 12 Pharm. Res. 1184-88 (1995) ("Niemiec"), which is incorporated by reference herein in its entirety. We have found that the presence of these liposomes in the compositions of this invention may enhance the therapeutic capabilities of some of the compositions of this invention.

Other preferable formulations may contain, for example, soybean milk or other liquid formulations derived directly from legumes or

other suitable plant. For example, such a formulation may contain a large proportion of soybean milk, an emulsifier that maintains the physical stability of the soybean milk, and, optionally a chelating agent, preservatives, emollients, humectants and/or thickeners or gelling agents.

Oil-in-water emulsions, water-in-oil emulsions, solvent-based formulations and aqueous gels known to those of skill in the art may also be utilized as vehicles for the delivery of the compositions of this invention.

The topical compositions useful in the present invention involve formulations suitable for topical application to skin. The composition may comprise the soy product and a cosmetically-acceptable topical carrier. The cosmetically-acceptable topical carrier may comprise from about 50% to about 99.99%, by weight, of the composition (e.g., from about 80% to about 95%, by weight, of the composition).

The compositions may be made into a wide variety of product types that include but are not limited to solid and liquid compositions such as lotions, creams, gels, sticks, sprays, shaving creams, ointments, cleansing liquid washes and solid bars, shampoos, pastes, powders, mousses, adhesive strips, and wipes. These product types may comprise several types of cosmetically acceptable topical carriers including, but not limited to solutions, emulsions (e.g., microemulsions and nanoemulsions), gels, solids and liposomes. The following are non-limitative examples of such carriers. Other carriers can be formulated by those of ordinary skill in the art.

The topical compositions useful in the present invention can be formulated as solutions. Solutions typically include an aqueous solvent (e.g., from about 50% to about 99.99% or from about 90% to about 99% of a cosmetically acceptable aqueous solvent).

Topical compositions useful in the subject invention may be formulated as a solution comprising an emollient. Such compositions preferably contain from about 2% to about 50% of an emollient(s). As used herein, "emollients" refer to materials used for the

prevention or relief of dryness, as well as for the protection of the skin. A wide variety of suitable emollients are known and may be used herein. See International Cosmetic Ingredient Dictionary and Handbook, eds. Wenninger and McEwen, pp. 1656-61, 1626, and 1654-55 (The Cosmetic, Toiletry, and Fragrance Assoc., Washington, D.C., 7th Edition, 1997) (hereinafter "INCI Handbook") contains numerous examples of suitable materials.

A lotion can be made from such a solution. Lotions typically comprise from about 1% to about 20% (e.g., from about 5% to about 10%) of an emollient(s) and from about 50% to about 90% (e.g., from about 60% to about 80%) of water.

Another type of product that may be formulated from a solution is a cream. A cream typically comprises from about 5% to about 50% (e.g., from about 10% to about 20%) of an emollient(s) and from about 45% to about 85% (e.g., from about 50% to about 75%) of water.

Yet another type of product that may be formulated from a solution is an ointment. An ointment may comprise a simple base of animal or vegetable oils or semi-solid hydrocarbons. An ointment may comprise from about 2% to about 10% of an emollient(s) plus from about 0.1% to about 2% of a thickening agent(s). A more complete disclosure of thickening agents or viscosity increasing agents useful herein can be found in the INCI Handbook pp. 1693-1697.

The topical compositions useful in the present invention may be formulated as emulsions. If the carrier is an emulsion, from about 1% to about 10% (e.g., from about 2% to about 5%) of the carrier comprises an emulsifier(s). Emulsifiers may be nonionic, anionic or cationic. Suitable emulsifiers are disclosed in, for example, INCI Handbook, pp.1673-1686.

Lotions and creams can be formulated as emulsions. Typically such lotions comprise from 0.5% to about 5% of an emulsifier(s). Such creams would typically comprise from about 1% to about 20% (e.g., from about 5% to about 10%) of an emollient(s); from about 20% to about 80% (e.g., from 30% to about 70%) of water; and from

about 1% to about 10% (e.g., from about 2% to about 5%) of an emulsifier(s).

Single emulsion skin care preparations, such as lotions and creams, of the oil-in-water type and water-in-oil type are well-known in the cosmetic art and are useful in the subject invention. Multiphase emulsion compositions, such as the water-in-oil-in-water type are also useful in the subject invention. In general, such single or multiphase emulsions contain water, emollients, and emulsifiers as essential ingredients.

The topical compositions of this invention can also be formulated as a gel (e.g., an aqueous gel using a suitable gelling agent(s)). Suitable gelling agents for aqueous gels include, but are not limited to, natural gums, acrylic acid and acrylate polymers and copolymers, and cellulose derivatives (e.g., hydroxymethyl cellulose and hydroxypropyl cellulose). Suitable gelling agents for oils (such as mineral oil) include, but are not limited to, hydrogenated butylene/ethylene/styrene copolymer and hydrogenated ethylene/propylene/styrene copolymer. Such gels typically comprise between about 0.1% and 5%, by weight, of such gelling agents.

The topical compositions of the present invention can also be formulated into a solid formulation (e.g., a wax-based stick, soap bar composition, powder, or a wipe containing powder).

The topical compositions useful in the subject invention may contain, in addition to the aforementioned components, a wide variety of additional oil-soluble materials and/or water-soluble materials conventionally used in compositions for use on skin, hair, and nails at their art-established levels.

The source of active compound to be formulated will generally depend upon the particular form of the compound. Small organic molecules and peptidyl fragments can be chemically synthesized and provided in a pure form suitable for pharmaceutical/cosmetic usage. Products of natural extracts can be purified according to techniques known in the art. Recombinant sources of compounds are also available to those of ordinary skill in the art.

In alternative embodiments, the topically active pharmaceutical or cosmetic composition may be optionally combined with other ingredients such as moisturizers, cosmetic adjuvants, anti-oxidants, bleaching agents, tyrosinase inhibitors and other known depigmentation agents, surfactants, foaming agents, conditioners, humectants, fragrances, viscosifiers, buffering agents, preservatives, sunscreens and the like. The compositions of this invention may also contain active amounts of retinoids (i.e., compounds that bind to any members of the family of retinoid receptors) and retinoid precursors such as retinol, including, for example, tretinoin, retinol, esters of tretinoin and/or retinol, synthetic retinoids such as those set forth in U.S. Patent No. 4,877,805, for example, and the like.

One of the problems encountered by many individuals who utilize retinoic acid-containing products is increased erythema caused by irritation, a common side effect of retinoid usage. We have found that, surprisingly, the combination of tretinoin and soybean extracts with trypsin inhibitory activity products, such as nondenatured soymilk powder, result in decreased skin redness when applied in combination with or simultaneously with retinoic acid. Preferably, the soy products are utilized in a topical composition containing from about 0.01 to about 50% soybean powder or soymilk powder, more preferably about 0.05 to about 20% soybean powder or soymilk powder and most preferably about 0.5 to about 5% soybean powder or soymilk powder.

The topically active pharmaceutical or cosmetic composition should be applied in an amount effective to reduce retinoid-induced irritation of mammalian skin. As used herein "amount effective" shall mean an amount sufficient to cover the region of skin surface where reduce retinoid-induced irritation is desired. Preferably, the composition is liberally applied to the skin surface such that, based upon a square cm of skin surface, from about 2 μl /cm^2 to about 200 μl /cm^2 of topically active agent is present when a reduction in irritation or redness is desired.

Natural extracts made directly from plants or botanical sources may be employed in the compositions of this invention in a concentration (w/v) from about 1 to about 99%. Fractions of natural extracts and naturally-derived protease inhibitors such as STI or BBI may have a different preferred range, from about 0.01% to about 20% and, more preferably, from about 0.5% to about 10% of the composition, and most preferably from 0.1% to about 2.5%. Of course, mixtures of the active agents of this invention may be combined and used together in the same formulation, or in serial applications of different formulations.

We have unexpectedly found that when topically active agents are topically applied to an animal's skin, a significant change in skin condition was achieved. Preferably, active agents of this invention are applied to the skin of a mammal at a relatively high concentration and dose (from about 0.005% to about 1% for compounds having high therapeutic indices such as natural and synthetic retinoids and related compounds; from about 20% to about 99% for liquid derivatives and extracts of botanical materials; and from about 0.1% to about 20% for dried extracts or fractions of natural extracts and naturally-derived protease inhibitors such as STI or mixtures thereof) between one and two times daily for a period of time until the skin evidences a change in skin condition. This may be for from about four to about ten weeks or more. Thereafter, once the change in skin condition has been achieved, a lower concentration and dose (from about 0.00001% to about 0.005% for compounds having high therapeutic indices such as natural and synthetic retinoids and related compounds; from about 10% to about 90% for liquid derivatives and extracts of botanical materials; and from about 0.01% to about 5% for fractions of natural extracts and naturally-derived protease inhibitors such as STI or mixtures thereof), of active ingredient may be applied on a less frequent time schedule, e.g., about once per day to about twice per week. The effects of the active agents of this invention are reversible, therefore, in order to maintain these effects, continuous

application or administration should be performed. The invention illustratively disclosed herein suitably may be practiced in the absence of any component, ingredient, or step which is not specifically disclosed herein.

Several examples are set forth below to further illustrate the nature of the invention and the manner of carrying it out, but do not serve to limit the scope of the methods and compositions of this invention.

Example 1: Preparation of naturally-derived products containing STI

Based on analytical testing, it has been determined that soybean milk and soybean paste are rich sources of soybean trypsin inhibitor.

To make soybean paste, soybeans were first soaked in deionized or purified water for several hours. The soybeans were ground after they were fully hydrated, with the addition of small quantities of water, if needed, to smoothen the paste. To make soybean milk, the same procedure was performed with the addition of more water. (The grinding process allows the soybean milk to be extracted). After collection, the soybean milk was filtered to remove any residual parts of the bean husk.

Soybean milk, soybean paste and miso were prepared to be used as naturally-derived materials that contain STI and are able to lighten skin color.

Example 2: Skin treatment formulations with soybean milk

In making the soybean milk, it was discovered that the rich emolliency of the milk would be desirable in a skin care formulation. Because water is used as the predominant ingredient of any oil-in-water emulsion, and in many other skin-care formulations we hypothesized that the soymilk could be used to substitute for the deionized water in such formulations. However, we expected that this type of formulation would not be physically stable due to the

immiscibility of the oil and water components of the soybean milk. Surprisingly, we found that this substitution of soybean milk for water was physically stable. Formulations utilizing soybean milk should contain between about 1% and about 99% of soybean milk, more preferably from about 80% to about 95% soybean milk. Preferably, this and similar formulations should include a viscosity builder in an amount from about 0% to about 5% (more preferably, from about 0.1 to about 2%), one or more emollients in an amount up to about 20% and/or emulsifiers in an amount from about 0.1% to about 10% (more preferably from about 3 to about 5%), and, optionally, a spreading agent in an amount from about 0 to about 5% (more preferably from about 1 to about 2%), a preservative, a chelating agent or a humectant. The preservative should be present in an effective amount in order to preserve integrity of the milk and maintain the composition's activity. Sufficient thickener should be present to impart body to the formulation without causing it to become so viscous that it would hinder spreadability, e.g., from about 0 to about 10%, more preferably from about 3 to about 5%. Sunscreen, antioxidants, vitamins other depigmenting agents and other skin care topical ingredients may also be incorporated into the compositions of this invention.

A particularly preferred example of a skin treatment formulation substituting soymilk for water is shown in table A below.

TABLE A

Ingredient	Function	% Wgt/Wgt
soybean milk	Vehicle, depigmenting	84.9%
aluminum starch octenyl succinate	viscosity builder	0.75%
cyclomethicone	spreading agent	2%
PEG 6-capric/caprylic triglycerides	emollient/emulsifier	3%
phenoxyethanol	preservative	0.75%
sucrose cocoate	emollient/emulsifier	1%
Na_2EDTA	chelating agent	0.1%

glycerin	humectant	2.5%
polyacrylamide; isoparaffin; laureth-7	thickener	5%

STI, soybean paste and other trypsin inhibitor-containing natural extracts can be incorporated into such formulations to provide increasing concentrations of the serine protease inhibitor. Use levels of the added active ingredient can range between 0.01% to 15% in a formulation. Other depigmenting agents, including PAR-2 inhibitors, tyrosinase inhibitors, hydroquinones, soy products, ascorbic acid and its derivatives, as well as other ingredients with skin care benefits could also be incorporated into this formulation.

Example 3: An Oil-in-water Emulsion skin treatment formulation

Two examples of a skin treatment formulation with oil-in-water emulsion are presented in Table B. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 3 of Table B. The therapeutic agents in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also listed in Table B. The deionized water content of these formulations could be replaced with soybean milk.

Table B

Phase	CTFA Name	Function	%W/W	%W/W	Ranges
OIL	Cetearyl Glucoside	Surfactant	1.4	1.4	0.1-2.8
	C12-15 Alkyl Benzoate	Surfactant	4.0	4.0	1-6
	Octyl Hydroxystearate	Emollient	1.0	1.0	0-5
	Dimethicone	Spreading Agent	1.0	1.0	0-5
	Cyclomethicone	Spreading Agent	1.0	1.0	0-5
	Cetyl Alcohol	Emollient	2.5	2.5	0-4
	Butylated Hydroxytoluene	Anti-oxidant	0.1	0.1	0-0.5
	Octyl Methoxycinnamate	Sunscreen	6.0	6.0	0-10
	Propylparaben	Preservative	0.5	0.1	0-0.5
	Vitamin E acetate	Anti-oxidant	0.5	0.5	0-0.5
	Tocopherol Acetate	Anti-oxidant	0.5	0.5	0-0.5
AQUEOUS	Glycerine	Humectant	3.0	3.0	0-20
	D-Pathenol	Pro-Vitamin	0.5	0.5	0-5
	Disodium EDTA	Chelator, whitening agent	0.1	0.1	0.01-1
	Methyl Paraben	Preservative	0.2	0.2	0-0.3
	Carbomer	Thickener	0.35	0.35	0-3
	Deionized Water or Soybean Milk	Carrier / Therapeutic agent	76.35	77.5	50-80
	STI or natural extract	Therapeutic Agent	1.0	0	0-15
	Other Therapeutic agents	Therapeutic Agent	0	0.25	0-1

To prepare this formulation, the ingredients of the lipid phase were combined and mixed at 85°C, and then cooled to 60°C. In a separate vessel, the carbopol was slowly added to the water or to the soybean milk. After mixing for ten minutes the rest of the aqueous phase ingredients were added and the mix was heated to 60°C. The two phases were then combined, mixed for ten minutes, and cooled to room temperature. Of course, one or more depigmentation agents may be combined within the same formulation, in this Example and in the following examples and other embodiments of the methods and compositions of this invention.

Example 4: Skin treatment Composition (Oil-in-Water Emulsion)

Two additional examples of an oil-in-water emulsion skin treatment formulation are presented in Table C. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 3 of Table C. The therapeutic agents in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also listed in Table C. The deionized water content of these formulations could be replaced with soybean milk.

Table C

CTFA Name	Function	%W/W	%W/W	Pref'd. Ranges
Ethanol	Solvent	12.0	12.0	5-20
Propylene Glycol	Solvent	3.0	3.0	1-10
Hydroxyethylcellulose	Thickener / Polymer	0.2	0.2	0-3
Acrylates/ C10-30 Alkyl Acrylate Crosspolymer	Thickener / Polymer	1.0	1.0	0-3
Panthenol (98%)	Pro-Vitamin / Humectant	1.5	1.5	0.1-3
Fragrance	Fragrance	0.5	0.5	0-0.5
Isohexadecane	Spreading Agent	4.0	4.0	0-5
Vitamin E acetate	Anti-oxidant	1.0	1.0	0-2
Sodium Hydroxide	Neutralizer	0.35	0.35	0.1-0.5
Glycerine	Humectant	3.0	3.0	0-20
Deionized Water or Soybean Milk	Carrier / Therapeutic Agent	72.2	71.95	60-80
Therapeutic agent	Therapeutic Agent	0	0.25	0-1
STI or natural extract	Therapeutic Agent	1.0	0	0-15

To prepare this formulation, the hydroxyethylcellulose was slowly added to the water or to the soybean milk and stir until completely dissolved. In a separate container the Acrylates/ C10-30 Alkyl Acrylate Crosspolymer was added and stir until completely dissolved. The content of the two containers was combined and mixed for 20 minutes. Vitamin E acetate was then added and mixed, following by the addition of Isohexadecane and Panthenol (98%). After mixing for five minutes the STI, or the natural extract, were added together with Propylene Glycol, and stirred for 5 minutes. Next, glycerine was added and the formulation was stirred for 20 minutes. Finally, the pH was adjusted with sodium hydroxide (for STI the range is 6-8.5).

Example 5: Skin Treatment Composition (Water-In-Oil Emulsion)

An example of a skin treatment formulation with water-in-oil emulsion is presented in Table D. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 4 of Table D. A similar formulation with a therapeutic agent is presented in column 5 of Table D. The therapeutic agents in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also listed in Table D. The deionized water content of these formulations could be replaced with soybean milk.

Table D

Phase	CTFA Name	Function	%W/W	%W/W	Pref'd Ranges
OIL	Mineral Oil	Emollient	25.0	25.0	40-80
	Sorbitan Monooleate	Surfactant	5.0	5.0	1-6
	Stearyl Alcohol	Emollient	25.0	25.0	20-60
	Dimethicone	Spreading Agent	1.0	1.0	1-5
	Cetyl Alcohol	Emollient	2.0	2.0	0.1-10
	Hydrogenated Lecithin	Anti-oxidant	3.0	3.0	0-10
	Parsol MCX	Sunscreen	3.0	3.0	0-10
	Propylparaben	Preservative	0.5	0.5	0.01-0.5
	Vitamin E acetate	Anti-oxidant	0.5	0.5	0.01-0.5
AQUEOUS	Glycerine	Humectant	3.0	3.0	0-20
	Methyl Paraben	Preservative	0.2	0.2	0.01-0.3
	Water or Soy Milk	Carrier / Therapeutic Agent	30.8	31.55	20-45
	STI	Therapeutic Agent	1.0	0	0-10
	Therapeutic agent	Therapeutic Agent	0	0.25	0-1

To prepare this formulation the stearyl alcohol and mineral oil were melted at 70°C. The other oil phase ingredients were added and the mixture heated to 75°C. The aqueous phase ingredients, which

have been previously dissolved in the bulk phase water or Soy Milk and warmed to 70°C, were then added and the mixture was stirred until it congealed.

Example 6: Skin Treatment Composition (Aqueous Gel)

Two examples of a skin treatment formulation with aqueous gel are presented in Table E. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 3 of Table E. The therapeutic agents in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also listed in Table E. The deionized water content of these formulations could be replaced with soybean milk.

Table E

CTFA Name	Function	%W/W	%W/W	
Octoxynol-13	Surfactant	0.2	0.2	0.05-0.5
2,4-Hexadienoic Acid	Preservative	0.1	0.1	0-0.3
Benzenemethanol	Preservative	1.0	1.0	0-2
Disodium EDTA	Chelator / Preservative	0.05	0.05	0.01-0.2
Ascorbic Acid	Anti-oxidant	0.1	0.1	0-0.2
Sodium Metabisulfite	Anti-oxidant	0.2	0.2	0-0.3
Carbomer	Thickener	1.5	1.5	0-3.0
NaOH %20 Soln.	Neutralizer	2.45	2.45	0.1-5
DEIONIZED Water or Soybean Milk	Carrier / Therapeutic Agent	93.4	94.15	85-98
STI or natural extract	Therapeutic Agent	1.0	0	0-15
Therapeutic Agent	Therapeutic Agent	0	0.25	0-1

To prepare this formulation, the Disodium EDTA, sodium metabisulfite and ascorbic acid were slowly added to the water or to the soybean milk and stir until completely dissolved. STI, natural extracts or therapeutic agents were then added and mixed slowly for five minutes. The speed of agitation was then increased and carbopol was added. The composition was mixed for 30 minutes or until the dispersion was free of "fish eyes", which are non-dispersed clear lumps, and heated to 50°C. In a separate container, the slurry phase was prepared by combining Octoxynol-13, 2,4-Hexadienoic acid, and Benzenemethanol and stirring ten minutes at 40-50°C. The slurry was then added slowly to the aqueous phase, mixed, and cooled to 45°C. 20% sodium hydroxide solution was used to pH the composition to pH of 7.0 (range is 5.5-8.5). This was mixed to homogeneity using agitation or sweep vessel.

Example 7: Solvent-based skin Treatment Composition

An example of a skin treatment formulation containing solvent is presented in Table F. A formulation with STI, where STI could be replaced with any naturally-derived serine protease inhibitor, or with any naturally-derived extract or fraction thereof containing serine protease inhibitors, is described in column 3 of Table F. The therapeutic agents in this composition could be replaced with similar compounds or with serine protease inhibitor or with any PAR-2 inhibitor materials having high therapeutic indices, whether derived synthetically or naturally, as the active ingredient. Suggested ranges for the ingredients in such formulations are also listed in Table F. The deionized water content of these formulations could be replaced with soybean milk

Table F

CTFA Name	Function	%W/W	Range
Ethanol	Solvent (1)	70	40-90
Propylene Glycol	Solvent (2)	29	1-40
Deionized Water	Carrier	q.s.	1-40
STI	Therapeutic Agent	0	0.01 - 50%
Therapeutic Agent	Therapeutic Agent	1 μM	0.00001 - 1

To prepare this formulation in accordance with parent application U.S. Serial No. 09/110,409 a serine protease inhibitor was dissolved in water. The ethanol and propylene glycol were mixed and combined with the aqueous solution containing the serine protease inhibitor.

Example 8: treatment of mice using Nondenatured Soy and Retinoic Acid shows reduced redness

RHJ/LE Hairless (Rhino) male mice, 5-7 weeks of age, were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were acclimated for one week, and then treated for 14 days, once/day,

with test material. Test material includes Renova® (0.05% Tretinoin, Ortho Neutrogena, CA), with and without 2.5 % Soymilk powder. Soymilk powder, of non-denatured soybean milk, is described in U.S. patent application Serial No. 09/698,454 filed October 27, 2000. Soymilk powder was obtained from DevonSoy Farms (Carroll, Iowa), and was mixed w/w into the tretinoin product using Polytron LS10-35 homogenizer. An untreated group served as a control for the study. Each test group contained 5 mice.

At the end of the treatment period, mice were visually observed for skin redness, and for reduced wrinkle appearance. Visual observations indicate that Tretinoin treated mice demonstrated skin redness, while the control, untreated mice were only slightly pink. Surprisingly, the combination of Tretinoin with the soymilk powder resulted in reduced skin redness, as indicated in Table G.

Table G

Treatment	**Skin Redness**
Untreated	1
Tretinoin	4
Tretinoin+Soy	2

Key: 1= light pink, 2= pink, 3= pink-red, 4= red

Visual observations also indicated that Tretinoin treated mice had reduced wrinkles. The addition of Soy to the Tretinoin did not change this effect, enabling Tretinoin to reduce wrinkles without interference (see Table H)

Table H

Treatment	**Wrinkles**
Untreated	3
Tretinoin	2
Tretinoin+Soy	2

Key: 0= no wrinkles, 1= few wrinkles, 2= moderate wrinkles, 3= severe wrinkles

This example demonstrates that the addition of non-denatured soy extracts to a tretinoin-containing product reduces the redness associated with retinoid treatment, without any negative effect on the retinoid activity.

Example 9: Treatment of mice using Nondenatured Soy and Retinoic Acid

Following visual observations, the mice described in Example 8 were sacrificed, and samples from their skin were evaluated histologically. H&E staining and histological analysis were performed using standard techniques as described in Sheehand and Hrapchak, 1980. Examination of the H&E stained skin sections revealed the following observations: Untreated Rhino mouse skin has large utriculi. The epidermis is very thin, and lines around the utriculi. No hair follicles are observed. Following the tretinoin treatment, the epidermis thickens, the utriculi disappear, and epidermal structures that look like hair follicles are produced. The skins treated with tretinoin plus soy had a histology profile very similar to that of the tretinoin treated skins. The utriculi disappeared, the epidermis thickened, and epithelial follicle-like structures were visible. Interestingly, these epithelial follicular structure looked "smoother" and somewhat healthier, relative to those of the tretinoin treated ones. This example demonstrates that the addition of non-denatured soy extracts into tretinoin products does not reduce the effectiveness of the retinoid, and possibly enhances the quality of the treated skin.

Example 10: Treatment of mice using Nondenatured Soy and Retinoic Acid

Skin samples from the mice of Example 8 were processed according to: Mezick JA, Bhatia MC, Capetola RJ, Topical and systemic effects of retinoids on horn-filled utriculus size in the

rhino mouse. A model to quantify "antikeratinizing" effects of retinoids., *J Invest Dermatol* 83: 2, 110-3, Aug, 1984. Epidermal sheets were separated as described, and the diameter of the utriculi was measured using computerized image analysis (Image Pro Plus version 4.5 from Media Cybernetics, Silver Spring, MD), CCD camera Hitachi KP-D50 and microscope Olympus BH-2). For each test group, the diameter of 50 epidermal utriculi was measured. A decrease in size of the utriculi versus control indicates a biologically active retinoid. As shown in Table I, tretinoin treated utriculi were reduced in size by about 63%. The tretinoin-soy treated group showed similar results, demonstrating, again, that the retinoid effect is not inhibited by the addition of soy, while benefits like redness reduction (Example 8) were added.

Table I

Treatment	**Utriculi diameter (μM +/- STD)**	**%reduction (relative to control)**
Control	94.7 +/- 6.9	-
Tretinoin	34.6 +/- 1.6	63.4
Renova+Soy	34.8 +/- 1.2	63.2

Example 11: Human Irritation Study

A 6-week human irritation and sensitization study on human was also conducted and completed. The results demonstrated a 34% reduction in irritation in cases in which a soy/retinol combination was applied to the skin. No sensitization was noted.

Method:

Approximately 200 subjects were patched intermittently with the formulation three times a week for a total of nine applications over a 3-week period. Sites were graded three times per week after each patch removal. After a 2-week rest period in which no test material was applied, a challenge patch of the formulation was

applied in on virgin site of the subjects. The test sites were graded after patch removal at 24, 74, 96 hr time periods and the results set forth below in Table J.

Table J:

Formulation	**Irritation Score**	**Irritation %**
Essence vehicle+0.1% Retinol	381	100%
Soy essence 5%+0.1% Retinol	255	66%

Example 12

RHJ/LE Hairless (Rhino) male mice, 5-7 weeks of age, were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were acclimated for one week, and then treated for 14 days, once/day, with test material. Test material includes Renova® brand tretinoin product(0.05% Tretinoin, available commercially from OrthoNeutrogena, CA), with and without 0.1% STI (Soybean Trypsin Inhibitor, Sigma-Aldrich Corp., St. Louis, MO). STI was mixed w/w into the Renova® product using a Polytron LS10-35 homogenizer. A vehicle-treated group served as a control for the study. Each test group contained 5 mice.

At the end of the treatment period, mice were visually observed for skin redness, and for reduced wrinkle appearance. Visual observations indicate that Renova® treated mice demonstrated skin redness, while the control, vehicle-treated mice were only slightly pink. Surprisingly, the combination of Renova® with STI resulted in reduced skin redness, as indicated in Table K.

Table K

Treatment	Skin Redness
Vehicle	1
Renova®	4
Renova®+STI	1

Key: 1= light pink, 2= pink, 3= pink-red, 4= red

Visual observations also indicated that Renova treated mice had reduced wrinkles. The addition of STI to the Renova® did not change this effect, enabling Renova to reduce wrinkles without interference (see Table L).

Table L

Treatment	Wrinkles
Untreated	3
Renova®	2
Renova®+STI	2

Key: 0= no wrinkles, 1= few wrinkles, 2= moderate wrinkles, 3= severe wrinkles

This example demonstrates that the addition of STI to a tretinoin-containing product reduces the redness associated with retinoid treatment, without any negative effect on the retinoid activity.

Example 13

Following visual observations, the mice described in Example 12 were sacrificed, and samples from their skin were evaluated histologically. H&E staining and histological analysis were performed using standard techniques as described in Sheehand and Hrapchak, 1980. Examination of the H&E stained skin sections revealed the following observations: Vehicle-treated Rhino mouse skin has large utriculi. The epidermis is very thin, and lines appear around the utriculi. No hair follicles are observed. Following the tretinoin treatment, the epidermis thickens, the utriculi disappear, and epidermal structures that look like epidermal follicular structures are produced. The skins treated with tretinoin combined with STI had a histology profile very similar to that of the tretinoin treated skins. The utriculi disappeared, the epidermis thickened, and epithelial follicular structures were visible. This example demonstrates that the

addition of STI into tretinoin products does not reduce the effectiveness of the retinoid.

Example 14

Skin samples from the mice of Example12 were processed according to: Mezick JA, Bhatia MC, Capetola RJ, Topical and systemic effects of retinoids on horn-filled utriculus size in the rhino mouse. A model to quantify "antikeratinizing" effects of retinoids., J Invest Dermatol 83: 2, 110-3, Aug, 1984. Epidermal sheets were separated as described, and the diameter of the utriculi was measured using computerized image analysis (Image Pro Plus version 4.5 from Media Cybernetics, Silver Spring, MD), CCD camera Hitachi KP-D50 and microscope Olympus BH-2). For each test group, the diameter of 50 epidermal utriculi was measured. A decrease in size of the utriculi versus control indicates a biologically active retinoid. As shown in Table M, Renova®-treated utriculi were reduced in size by about 70%. The tretinoin-STI treated group showed similar results, demonstrating, again, that the retinoid effect is not inhibited by the addition of STI, while benefits like redness reduction (as set forth in Example 12) are added.

Table M

Treatment	Utriculi diameter (μM +/- STD)	% reduction (relative to control)
Control	94.7 +/- 6.9	-
Tretinoin	34.6 +/- 1.6	63.4
Renova®+STI	29.4 +/- 0.6	68.9